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*Brief*  
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of

ECKSTEIN *et al.*

Serial No. 08/936,657

Filed: August 16, 1995

For: **MODIFIED RIBOZYMES**



Group Art Unit: 1635

Examiner: LeGuyader, J.

Atty. Docket No.: 00-838A (228/231)

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NOV 20 2000

TECH CENTER 1600/2900

**BRIEF ON APPEAL**

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For: **MODIFIED RIBOZYMES**

**BRIEF ON APPEAL AND  
PETITION FOR EXTENSION OF TIME**

Honorable Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

An original and three copies of this appeal brief are submitted along with the large entity fee of three hundred ten dollars (\$310.00) for filing an appeal.

A petition to revive this application was granted on August 15, 2000. The two-month period for filing an appeal brief runs from the date of the granted petition, *i.e.*, until October 15, 2000. Appellants hereby petition for a one-month extension of time for filing an Appeal Brief. Accordingly, it is respectfully requested that an extension of time until November 15, 2000 be granted. The large entity extension fee of one hundred ten dollars (\$110.00) is attached. In the event of any variance between any of the amounts enclosed and the Patent and Trademark Office charges, please charge or credit any difference to our Deposit Account No. 13-2490.

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## **REAL PARTY IN INTEREST**

The real party in interest is Ribozyme Pharmaceuticals, Inc., which is the licensee of Max-Planck-Gesellschaft zur Forderung der Wissenschaften E.V., to whom this invention is assigned.

## **RELATED APPEALS AND INTERFERENCES**

Applicant is aware of no related appeals or interferences concerning this application.

## **STATUS OF CLAIMS**

Claims 44-57 are finally rejected. Claims 1-43 have been canceled. A clean set of the pending claims 44-57 is attached in Appendix A.

## **STATUS OF AMENDMENTS**

There are no outstanding amendments.

## **SUMMARY OF THE INVENTION**

The invention is directed to methods of cleaving a target RNA molecule by combining the target RNA molecule with a catalytic RNA molecule under cleavage conditions. Specification at page 13, lines 27-33. The catalytic RNA molecule comprises at least one modified nucleoside. The modified nucleoside comprises a modifier group, selected from the group consisting of halo, sulfhydryl, azido, amino, monosubstituted amino, and disubstituted amino groups. The modifier group replaces a hydroxy group at the ribose sugar 2'-position. Specification at page 4, lines 21-26. The modifications can be made to any type of ribozyme, such as a hammerhead or hairpin ribozyme. Specification at page 5, lines 7-12. The target RNA can be cellular or foreign genetic material, such as a viral material. Specification at page 13, lines 27-33.

## ISSUE

The issue presented to the Board for review by this appeal is:

Whether claims 44-57 are unpatentable under 35 U.S.C. §112, first paragraph, as not enabled such that one of skill in the art could not make and/or use the invention in light of the patent specification.

## GROUPING OF THE CLAIMS

Claims 44-57 stand or fall together.

## ARGUMENT

Claims 44-57 stand rejected as not enabled under 35 U.S.C. §112, first paragraph. The Examiner asserts that the specification fails to provide any specific or particular guidelines for engineering and delivering the claimed modified ribozymes, such that the ribozymes will bind and cleave a target in cells. The Examiner asserts that the specification provides no guidance as to what modifier groups can be used or at what positions in ribozyme motifs such modifiers can be tolerated so as to provide for a functional ribozyme. The Examiner also asserts that there is no guidance for the determination of ribozyme cleavage sites in any target RNA.

However, as detailed *infra*, the specification thoroughly describes the modified ribozymes of the instant invention, including a description for preparing such ribozymes. Moreover, the specification discusses the use of such modified ribozymes *in vitro* and *in vivo*. Thus, the claims are enabled by the specification, and the rejection must be reversed.

**A. The specification describes the modified ribozymes and how to make them.**

The specification thoroughly describes the structure of the claimed modified ribozymes and how to make them. For example, at *inter alia*, page 5, line 24 through page 10, line 18, the specification teaches various forms of the claimed catalytic RNA molecules and describes various modifications useful in constructing the claimed modified ribozymes. The specification also describes in detail how to synthesize the claimed modified ribozymes, for example, by automated chemical synthesis or transcriptional incorporation of appropriate nucleic acids. *See, e.g.*, page 10, line 19 through page 13, line 26. A specific example of synthesis of modified ribozymes of the invention is demonstrated in Example 1, at page 15 of the specification.

The specification also teaches various types of modifier groups that can be used in the claimed ribozymes and describes the various positions within a ribozyme motif where the modifier groups can be added. The claims specify, and the specification teaches, that the modifier groups are selected from the group consisting of halo, sulfhydryl, azido, amino, monosubstituted amino, and disubstituted amino groups. *See, e.g.*, specification at page 4, lines 23-24.

The specification expressly teaches that a modified ribozyme of the invention comprises at least one modified nucleoside where the hydroxy group at the 2' position of ribose is replaced by a modifier group, and also teaches the location of the modified groups within the claimed ribozyme types. *See, e.g.*, specification at page 8, line 6-8 and the Examples. The specification further teaches that embodiments of the invention may comprise, for example, an RNA molecule wherein all nucleosides of a particular kind (*i.e.*, either adenosine, guanosine, cytidine, or uridine) contain modified sugars, while the remaining nucleosides contain unmodified sugars. *See, e.g.*, specification page 8, line 3 through page 9. The specification goes on to list many other embodiments of the

invention, demonstrating the positions or locations of the modifier groups in the claimed ribozymes.

*See, e.g.*, specification page 8, line 6 to page 9, line 13.

Still further, the specification teaches one skilled in the art how to determine if a particular modification affects the catalytic activity of a ribozyme. Example 4 at page 24-26 shows that, by determining and comparing  $k_{cat}/K_m$  values for unmodified and modified ribozymes, a person skilled in the art can quantitate the affects of 2'-hydroxy modification on the catalytic activity of a ribozyme. Therefore, one skilled in the art could modify a ribozyme and determine the affect of the modification, without undue experimentation, given the teachings of the specification.

Finally, the specification and prior art also provide guidance to one of skill in the art for the determination of ribozyme cleavage sites in target RNA. Ribozymes are designed such that portions of the ribozyme are complementary to the target RNA. The structure and conserved regions of ribozymes, such as hammerhead and hairpin ribozymes, are described in detail in the background section of the specification. *See, e.g.*, specification page 1, line 1 to page 3, line 24. The specification also provides working examples of how to design ribozymes for a specific target. *See, e.g.*, Examples 1 to 3. Further, it was well known to those of skill in the art, at the time the invention was filed, how to design and target ribozymes to specific cleavage sites of mRNA molecules. The present application also teaches many such examples of the successful design of ribozymes in the prior art. *See, e.g.*, citations at page 2, line 2 to page 3, line 24. Additionally, since the filing of the instant application, a multitude of examples of the successful design and synthesis of the ribozymes for specific targets have been accomplished by those of skill in the art. Just a few of these many examples are described in paragraphs 9-14 of the declaration of Dr. Dan T. Stinchcomb ("the Stinchcomb declaration"), made of record in the response filed May 27, 1999, and attached as

Appendix B. This situation further corroborates that one of skill in the art, given the teachings of the specification and the state of the prior art, could successfully design and synthesize ribozymes that are specific for a given target without undue experimentation.

**B. The specification also describes how to use the modified ribozymes.**

The specification (at page 3, lines 28-30) teaches *in vivo* delivery of the modified ribozymes of the invention into target cells by:

- (a) exogenous delivery of a preformed synthetic RNA; [and]
- (b) endogenous transcription of a ribozyme-coding gene located on a plasmid.

One of skill in the art could deliver ribozymes to cells without undue experimentation based on the teachings of the specification and the state of the knowledge at the time the invention was made. Methods for delivering oligonucleotides to cells, as taught in the specification, were known in the art at the time of filing. Therefore, one of skill in the art would reasonably expect success in delivery of ribozymes to cells.

Additionally, the Stinchcomb declaration demonstrates the successful delivery of modified ribozymes *in vivo* following the suggestions of the specification. At paragraph 12 of the declaration, a study is presented where modified ribozymes in saline are delivered by injection into the synovium of rabbit knees *in vivo*. The modified ribozymes accumulate intact in synovial tissue and specifically cleave target mRNAs *in vivo*. Paragraph 14 of the declaration presents a study where modified ribozymes in saline were delivered by submandibular injection into newborn mice *in vivo*. The modified ribozymes specifically cleaved target mRNAs *in vivo*.

Pursuant to the teachings of the specification, delivery of modified ribozymes of the invention could be accomplished by one of skill in the art, as taught in the specification without



undue experimentation. As stated in the Stinchcomb declaration at paragraph 7: “[t]here are a number of additional approaches described in the art that can be readily adopted to deliver ribozymes into a cell. The choice of delivery system can be readily made by carrying out routine and standard experiments well known in the art.” Thus, it would not require undue experimentation for those of skill in the art, at the time the invention was made, to deliver the therapeutic ribozymes of the instant invention *in vivo* and *in vitro*. As explained by the Federal Circuit in *In re Wands*, 8 U.S.P.Q2d 1400, 1404 (Fed. Cir. 1988):

The key word is “undue” and not “experimentation”. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.”

As expressed by Dr. Stinchcomb, those in the art would be able to deliver the instant modified ribozymes *in vivo* and *in vitro* using known methods, and thus is would not require undue experimentation to use these ribozymes.

The specification also describes in detail how to use the modified ribozymes *in vitro* and *in vivo*. The specification teaches that the modified ribozymes are useful as:

therapeutic agents, especially for the specific cleavage of viral or other foreign genetic material or transcripts from viral or other foreign genetic material, or as a biocatalyst in biochemical or biotechnological processes. (Specification, page 13, lines 29-33.)

The reference to “therapeutic agents” identifies an *in vivo* application and the reference to “biocatalyst” identifies an *in vitro* application. These uses encompass both *in vivo* and *in vitro* applications. The examples of the specification demonstrate the specific cleavage of targets by the ribozymes of the invention *in vitro*.

Furthermore, as discussed in the specification, the use of ribozymes as therapeutic agents *in vivo* was envisioned by those in the art prior to the filing of the instant application. *See, e.g.*, specification, page 2, line 10 through page 3, line 10. The prior art envisioned that ribozymes could effectively and specifically cleave their targets in cells. *See, e.g.*, specification, page 2, line 10 through page 3, line 10. However, prior to the present invention, scientists were unable to produce and deliver ribozymes with sufficient stability to accomplish that objective. The present application is directed to the delivery and use of these modified ribozymes created by the present invention. The modifications of this invention are described and claimed in the application. The present invention is focussed not on the delivery and use of oligonucleotides, which was generally known, but rather is directed to the delivery and use of ribozymes having the modifications disclosed and claimed herein. Both the *in vitro* examples provided in the specification and the state of the prior art, is reasonably predictive of the ability of ribozymes to cleave targets *in vivo* and *in vitro*.

The Stinchcomb declaration further provides many examples of specific cleavage of targets *in vivo* with modified ribozymes. For example, as discussed *supra*, paragraph 12 of the declaration describes a study where modified ribozymes are delivered by injection into the synovium of rabbit knees and specifically cleave target mRNAs *in vivo*. Paragraph 14 of the declaration presents a study where modified ribozymes were delivered by submandibular injection into newborn mice and specifically cleaved target mRNA *in vivo*. Both these studies used exogenous delivery of the ribozymes as taught by the specification. *See* specification, page 3, lines 28-30. The Stinchcomb declaration also discloses experiments presented in Christoffersen & Marr (Exhibit 1 of the declaration), wherein DNA encoding a ribozyme designed to cleave the mRNA of the H-*ras* gene was cloned into a mammalian expression vector and transfected into human bladder carcinoma EJ

cells. *See*, page 2031, last paragraph through page 2032, first full paragraph. The cells were injected by transurethral implantation into mice to recapitulate the invasive potential of bladder carcinoma.

The EJ cell clones transfected with a ribozyme expressing vector showed a dramatic reduction in the malignant phenotype in mice compared to controls. This study demonstrates that ability of ribozymes to specifically cleave targets *in vivo* where the ribozymes are delivered endogenously, as taught by the specification. *See, e.g.*, page 3, line 25-30. Therefore, *in vivo* use of the modified ribozymes of the invention could be accomplished by one of skill in the art, as taught in the specification, without undue experimentation as demonstrated by experiments described in at least paragraphs 4, 12, and 14 of the Stinchcomb declaration.

Other examples of the successful cleavage of target sequences *in vivo* and in cell culture are presented in the Stinchcomb declaration. For example, paragraph 11 of the Stinchcomb declaration describes the delivery of modified ribozymes targeted against HIV into CD4+ cells as complexes with calcium phosphate. The ribozymes are shown to inhibit HIV replication in these cells. Paragraph 10 describes the administration of *c-myb* cleaving modified ribozymes to smooth muscle cells as complexes with a cationic lipid. These ribozymes were shown to effectively cleave *c-myb* and inhibit smooth muscle cell proliferation. As explained in the declaration, such ribozymes could potentially be delivered locally to vessel walls immediately following angioplasty procedures to reduce the incidence of restenosis that occurs after coronary angioplasty. Paragraph 9 describes experiments where *MDR-1* cleaving modified ribozymes were administered to human pleural mesothelioma cells with a cationic lipid. *MDR-1* is known in the art to encode a phosphoglycoprotein that can cause multiple drug resistance in cancer cells. As explained in the declaration, the ribozymes were shown to reduce *MDR-1* gene products and increase sensitivity of

the cells to an anti-neoplastic drug. In all of these well-recognized model systems modified ribozymes are capable of specifically cleaving their target RNAs. As shown in the examples, the ribozymes were expressed in these cells, and cleaved the target RNA in cells.

The Stinchcomb declaration observes that “cell culture and animal data showing efficacy of chemically-modified ribozymes is reasonably predictive of their utility as a therapeutic agent.” *See* paragraphs 5 and 15. The information presented in this declaration supports the position that efficacy of ribozymes in an appropriate cell culture or animal models will be readily accepted by a person skilled in the art to be reasonably predictive of the ability of ribozymes to cleave target sequences in cells and *in vivo*.

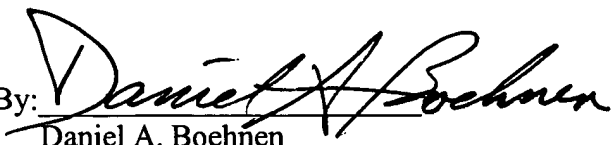
Therefore, the specification teaches one of skill in the art how to use the claimed modified ribozymes to cleave a target RNA *in vivo* and *in vitro* without undue experimentation.

**Summary**

On the basis of the foregoing and in view of the arguments presented herein, reversal of each and every rejection is appropriate.

Respectfully submitted,

Date: November 15, 2000

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## APPENDIX A

### Claims

44. A method of cleaving a target RNA molecule comprising the step of providing said target RNA molecule with a catalytic RNA molecule under conditions suitable for said catalytic RNA molecule to cleave said target RNA molecule, wherein said catalytic RNA molecule comprises at least one modified nucleoside, said modified nucleoside comprising a modifier group, selected from the group consisting of halo, sulfhydryl, azido, amino, monosubstituted amino and disubstituted amino groups replacing a hydroxy group at the ribose sugar 2'-position.
45. The method of claim 44, wherein said modifier group is a halo group.
46. The method of claim 44, wherein said modifier group is an amino group.
47. The method of claim 44, wherein said modifier group is a monosubstituted amino group.
48. The method of claim 44, wherein said modifier group is a disubstituted amino group.
49. The method of claim 44, wherein said modifier group is an azido group.
50. The method of any of claims 44-49, wherein said catalytic RNA molecule is a hammerhead ribozyme.
51. The method of any of claims 44-49, wherein said catalytic RNA molecule is a hairpin RNA.
52. The method of any of claims 44-49, wherein said target RNA molecule is a foreign genetic material.
53. The method of claim 50, wherein said target RNA molecule is a foreign genetic material.
54. The method of claim 51, wherein said target RNA molecule is a foreign genetic material.
55. The method of any of claims 44-49, wherein said target RNA molecule is a viral material.
56. The method of claim 50, wherein said target RNA molecule is a viral material.
57. The method of claim 51, wherein said target RNA molecule is a viral material.